

The first gynandromorph of the Neotropical bee *Megalopta amoena* (Spinola, 1853) (Halictidae) with notes on its circadian rhythm

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Abstract

Gynandromorphy is an anomaly that results in an organism phenotypically expressing both male and female characteristics. Here we describe the first gynandromorph of the bee species *Megalopta amoena* (Spinola, 1853) (Halictidae, Augochlorini) and the second record of this anomaly within the genus *Megalopta*. Additionally, we analyzed the bee's circadian rhythm, which has never before been quantified for a gynandromorph. The gynandromorph showed a deviant activity pattern; it was intermediate between that of the male and female *M. amoena*. Our results imply that the brains of bilateral gynandromorphs may have mixed sex-specific signaling. Based on four days of recording, the gynandromorph circadian rhythm was shifted earlier in the day relative to the male and female *M. amoena*, and it exhibited intensity similar to the female.

Keywords

Sweat bee, development, morphology, Augochlorini, gynandromorph, circadian rhythm

Introduction

Gynandromorphy is a rare occurrence in which an organism presents both male and female phenotypes (Wcislo et al. 2004a). Gynandromorphs have been reported in at least 140 bee species, which is only 0.7% of all bee species (Alvarez et al. 2019, Prashantha et al. 2019, reviewed by Wcislo et al. 2004a, Michez et al. 2009, Hinojosa-Díaz et al. 2012). This phenomenon may offer insight into the evolution of specialized morphological traits, such as male-like morphology of female brood-parasitic bee lineages (Wcislo et al. 2004a), the modified morphology of social insect castes (Yang and Abouheif 2011), and novel methods of reproduction (Aamidor et al. 2018). Gynandromorphs are usually unnoticed until specimens are in museum collections, meaning that the behavior and physiology of these bees are seldom studied (Michez et al. 2009, Sampson et al. 2010, Ugajin et al. 2016, Matsuo et al. 2018). Here we report the first gynandromorph discovered in the bee species *Megalopta amoena* (Halictidae, Augochlorini); only one has been previously reported from the genus *Megalopta* (*M. genalis* Meade-Waldo, 1916; Wcislo et al. 2004a).

When the gynandromorph was discovered as a newly eclosed adult, we were engaged in a separate study of the circadian rhythms of *Megalopta* bees (Krichilsky and Vega-Hidalgo et al. unpublished data). We tested the circadian rhythm of this individual using an automated activity monitoring system (Giannoni-Guzmán et al. 2014). The few previous studies of live gynandromorphs have reported data on nesting behavior (Michez et al. 2009), courtship (Michez et al. 2009, Sampson et al. 2010, Matsuo et al. 2018), and gene expression (Ugajin et al. 2016, Matsuo et al. 2018). This is the first report of circadian activity monitoring in a gynandromorph.

Circadian rhythms are ubiquitous in animals and serve to synchronize behavior, physiology, and ecological interactions with the external environment (Dunlap et al. 2004). In bees and other pollinators, modifications of circadian rhythms may allow insects to coordinate their foraging with the timing of floral resource availability (Bloch et al. 2017). The genus *Megalopta* has a bimodal foraging period, occurring in two narrow temporal windows of approximately 90 minutes after sunset and before sunrise, when it is nearly dark (Kelber et al. 2006, Smith et al. 2017).

Methods

Gynandromorph collection

The nest containing the gynandromorph was collected midday GMT-5 on April 24th, 2018 by KH on the Drayton trail in the neotropical forest of Barro Colorado Island, Panamá (BCI; 9°09'N, 79°51'W). The nest was collected by plugging the entrance with cotton wool, placing the nest in a bag, and opening the nest in the lab under a mesh tent. Then unrecognized as a gynandromorph, the individual was in a closed brood cell. Based on the time between discovery and emergence (26 days) and the development time of the congener *M. genalis* (Smith et al 2009, Kapheim et al 2011), it was likely at the

larval stage when collected. The brood cell containing the gynandromorph was put in an incubator to develop until adult emergence with other brood from the nest at 25 °C with 70% humidity, which approximates ambient conditions in the forest understory (Windsor 1990). The adults found in the nest were released. The individual was not monitored during development and not noticed as a gynandromorph until the adult stage. Upon eclosion, we weighed the gynandromorph and measured its intertegular and interocular distances using calipers. The bee was identified as *M. amoena* based on the lack of longitudinal rugulosity on the basal area of the metapostnotum, also known as the basal area of the propodeum (Engel 2006, Santos and Melo 2015). See Wcislo et al. (2004b) for a description of the nesting biology of *M. amoena*, then classified as *M. ecuadoria*; the species has also been previously classified with nine other names (Santos and Melo 2015).

Circadian rhythm

The circadian rhythm was quantified using a Locomotor Activity Monitor (LAM), modified from the *Drosophila* Activity Monitor system (DAM) (Trikinetics Inc., Waltman, MA, USA), following Giannoni-Guzmán et al. (2014). After being weighed and measured, the gynandromorph, an adult female, and a male of *M. amoena* were each placed in a modified 15ml centrifuge tube on May 20th, 2018 and put inside of the LAM and fed with a 50:50 honey:water solution with activity automatically recorded until the gynandromorph's death on May 24th, 2018. The activity monitor measured the number of times, per 15 minute interval, that each bee crossed the laser located in the middle of their tube. The LAM was housed in constant darkness (dark-dark cycle), to test the endogenous circadian rhythm, in an incubator with the same conditions as outlined above. We used the packages *damr* v0.3.4 and *ggetho* v0.3.4 of the rethomics workflow (Geissmann et al. 2019) to calculate the actograms in R v3.6.1 (R core team 2019).

We calculated activity percentage and intensity measures. The activity percentage was calculated as the percentage of activity across a day in the 15 minute intervals. If a bee did not cross the laser during an interval, it was recorded as nonactive, whereas if the bee did cross the laser at least one time, it was taken as an active interval. The activity percentage was the percentage of active intervals in a day, this measure did not take into account the intensity of the activity at each interval. The intensity was calculated by taking the highest number of laser crossings per interval in a given day. We report the average and standard deviation of the activity percentage and intensity across days. For the activity percentage and intensity measurements, we included only the second and third days to account for habituation in the first day and for the male and gynandromorph's early death.

Morphology

The terminology for external morphology follows Eickwort (1969) and Michener (2007), with modifications by Santos and Melo (2015).

Photography

A Keyence VHX-5000 digital microscope was used at 100–200× magnification to take images of the specimen. Images were edited and plates were prepared using Adobe Photoshop CC 2017.

Material deposited

The gynandromorph is now deposited at the Instituto Smithsonian de Investigaciones Tropicales (Smithsonian Tropical Research Institute) – Synoptic Insect Collection (STRI-ENTO) in Balboa, Ancón, Panamá. The *M. genalis* gynandromorph found by Wcislo et al. (2004a) was deposited in that same collection.

Results

Nest collection

The gynandromorph eclosed on 19 May, 2018. Its weight upon eclosion was 46.8 mg, the interocular distance was 1.35 mm, the intertegular distance was 1.10 mm, the metasoma was 1.6 mm long, and the overall body length was 4.03 mm. Upon collection, the nest contained two adult females, and three immature individuals, one of which was female, one male, and one gynandromorph (Table 1). The sex of the immature individuals was determined upon eclosion, as they were in an incubator in the sealed brood cells in which they were found. The presence of two adult females indicates a social nest where one was the queen and the other a daughter (Wcislo 2004b, Smith et al. 2019), but we did not dissect ovaries to confirm this. The other two immature individuals eclosed on 26 April, 2018 (male) and 3 May, 2018 (female).

Morphology

The phenotype expressed in the head has a distinct bilateral split of male and female characters (Figure 1A). The side with male characters is on the left, and the side with female characters is on the right, hereinafter referred to as the male side and the female side. The antenna on the male side has 11 flagellomeres, each with a length of 0.34 mm and a scape length of 0.71 mm. The antenna on the female side has 10 flagellomeres, each with a length of 0.23 mm and a scape length of 1.33 mm (Figure 1A). The mandible on the male side is weak, whereas the mandible on the female side is strong and bidentate with supplementary teeth (Figure 1B). The gena is not enlarged on the male side, but is on the female side in order to accommodate the larger mandibular muscu-

Table 1. Information on the contents of the nest collected by Kate Hunter (KH) from Drayton trail, BCI.

Species	Nest	Date Nest Collected	Location	Sex	Status	Eclosion Date
<i>M. amoena</i>	375	April 24 th , 2018	Drayton trail, BCI	Female	Adult	N/A
	375	April 24 th , 2018	Drayton trail, BCI	Female	Adult	N/A
	375	April 24 th , 2018	Drayton trail, BCI	Male	In cell- immature	April 26 th , 2018
	375	April 24 th , 2018	Drayton trail, BCI	Female	In cell- immature	May 3 rd , 2018
	375	April 24 th , 2018	Drayton trail, BCI	Gynandromorph	In cell- immature	May 19 th , 2018

lature characteristic of females (Figure 1C, D). The clypeal punctures are continuous, moderately dense, and separated by approximately one puncture width (Figure 1B). Ridges are not well defined on the right side of the head.

The posterior upper margin of the metepisternum was modified into a conspicuously large process covered with velvety pilosity (Santos and Melo 2015), it appeared similar on both sides. The hindleg on the female side is more hairy with a more robust femur and tibia, and curved apical spines on the femur (Figure 1G). The hindleg on the male side is less hairy with a more slender femur and tibia, and straight apical spines on the femur (Figure 1F).

The metasoma has bilateral asymmetry, split between male and female characters. This split was visible on the sterna, with the female side showing the metasomal scopa hairs on three fourths of the surface, used for pollen collection (Figure 1I). The male side showed few ventral abdominal hairs and S3 is mostly flat and lacking a mid longitudinal sulcus as in Santos and Melo 2015 (Figure 1E, I). There was no obviously visible asymmetry on the terga. The gynandromorph had a sting on the terminus of the metasoma, pointed outward from the female side of the body (Figure 1H). We did not dissect the specimen to observe internal anatomy, however the position of the sting indicates a mixed arrangement of both sexes.

Circadian rhythm

We compared activity of the 3 specimens in 24 hour darkness (dark:dark) for four days. The male and female activity rhythms are more similar to each other than they are to the gynandromorph (Fig. 2). The gynandromorph was active from 00 to 06 hours during days two to four, whereas the male and female of *M. amoena* showed almost no activity during that time. The gynandromorph's highest activity peak occurred in the interval from 06 to 12 hours. The female's highest activity peak was also from 06 to 12 hours. The male showed lower activity peaks than the other two bees, with the highest peak during the first day between 18 and 24 hours. The gynandromorph's activity percentage was $34 \pm 4\%$ per day, the female was $32 \pm 3\%$, and the male was $39 \pm 2\%$. Concerning the activity intensity, the gynandromorph averaged 91 ± 19.80 , the male 31.00 ± 2.83 , and the female 101.50 ± 43.13 .

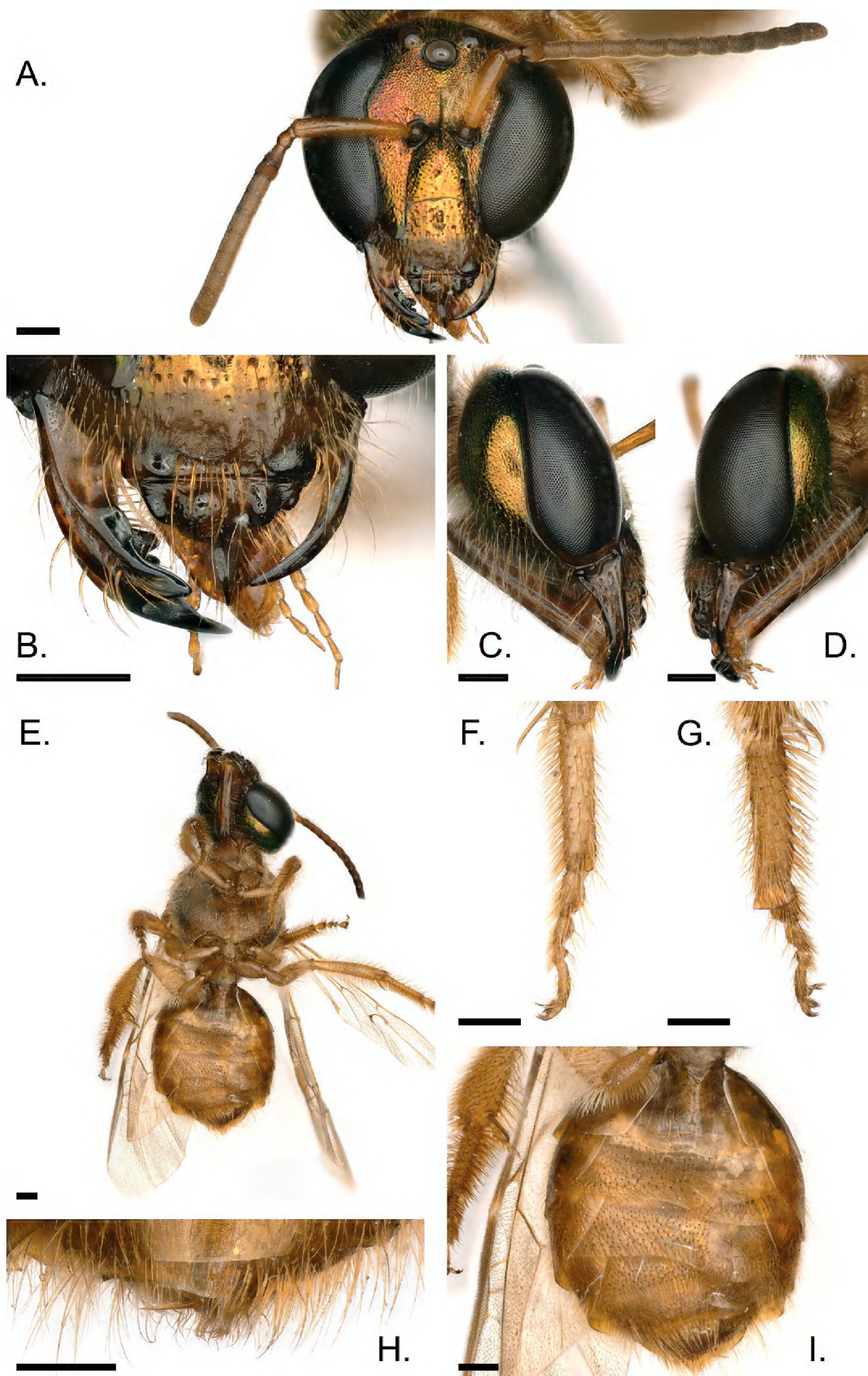


Figure 1. **A** Frontal view of the head showing the bilateral split between sexes (female left side of image, male right side) **B** detail of female (left) and male (right) mandibles and labrum, frontal view. Lateral view of the **C** male and **D** female genae and mandibles **E** ventral view of gynandromorph (female left side of image, male right side). Femur, apical spines, and tibia of **F** male and **G** female hindlegs **H** dorsal view of stinger **I** ventral view of metasoma (female left side of image, male right side). Scale bars: 500 μm .

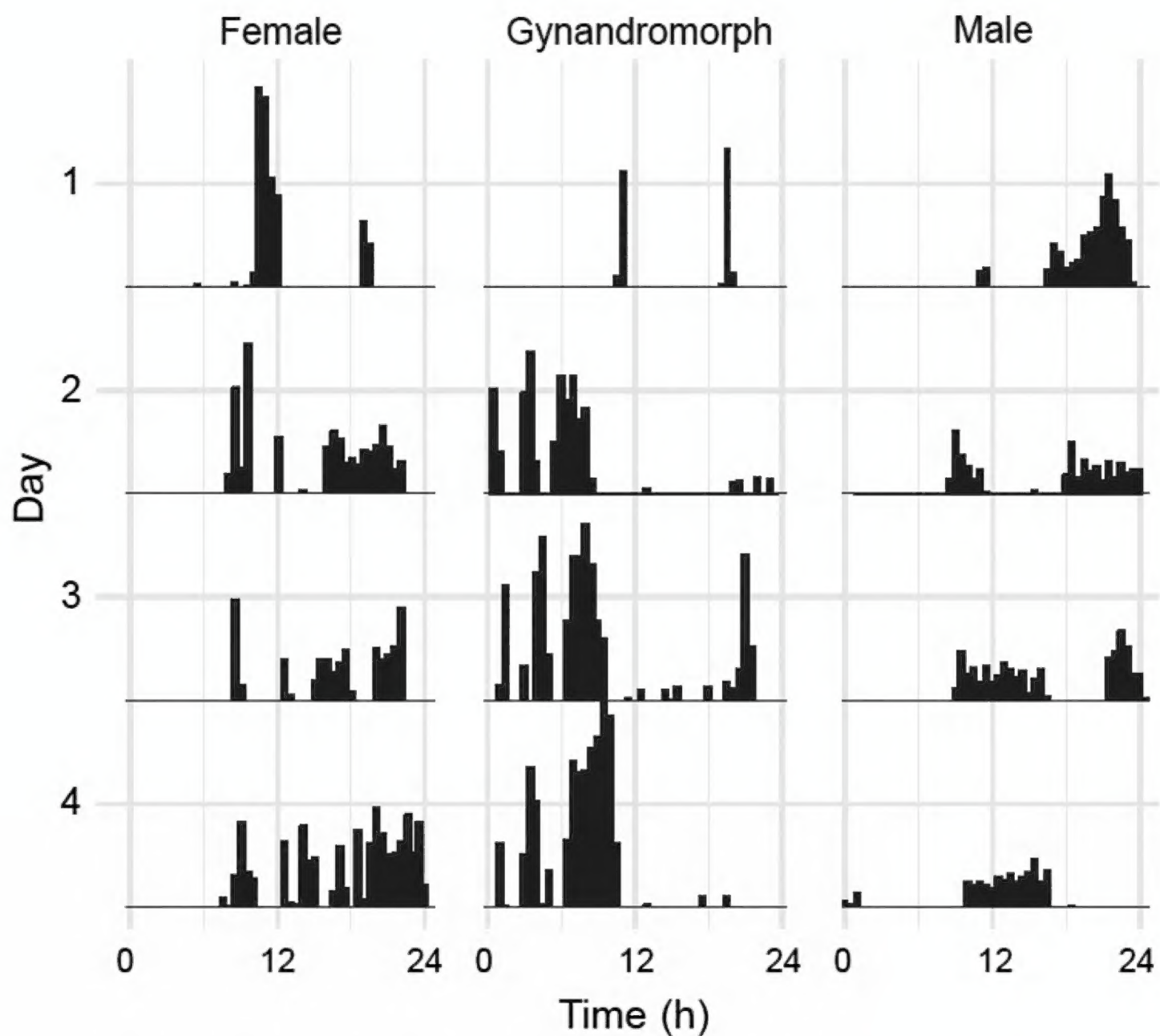


Figure 2. Single-plot actogram of four days of activity in dark-dark conditions showing a female, the gynandromorph, and a male *M. amoena*. Each bar represents the amount of times that the bee crossed the laser in a 15 minute interval. White space indicates lack of activity.

Discussion

Morphology

The gynandromorph of *M. amoena* reported here and that of *M. genalis* (Wcislo et al. 2004a), are the only two discovered for the genus, which occurs throughout the neotropics (Engel 2006, Santos and Melo 2015). Both gynandromorphs were discovered in the course of approximately 20 years of intensive field collections of *Megalopta* bees on BCI, Panamá (e.g. Wcislo et al. 2004b, Smith et al. 2019). This suggests that gynandromorphy is quite rare in the genus. Additionally, this finding is the fourth species of the tribe Augochlorini with a recorded gynandromorph (Alvarez et al. 2014, Engel and Hinojosa-Díaz 2011, Wcislo et al. 2004a). All four Augochlorini gynandromorphs were bilaterally split in the head; the two *Megalopta* gynandromorphs had

male characters on the left side and female on the right (Wcislo et al. 2004a), whereas the *Thectochlora alaris* (Vachal 1904) and *Auglochloa amphitrite* (Schrottky 1909) were the opposite with male characters on the right side and female characters on the left (Alvarez et al. 2014, Engel and Hinojosa-Díaz 2011). Most other recorded gynandromorphs from the family Halictidae are partially bilateral, with male and female expression on the heads and variable phenotypic expression in the mesosoma and metasoma (Wcislo et al. 2004a, Michez et al. 2009, Hinojosa-Díaz et al. 2012).

The gynandromorph of *M. amoena* is of the bilateral type (Michez et al. 2009). The head is approximately equal with respect to the bilateral split. We determined a bilateral split of the mesosoma based on leg morphology but not the metepisternum. The metasoma is majority female, based on the distribution of metasomal scopa hairs. Cockerell (1911) when describing a new genus, then *Androgynella*, hypothesized that a reduced scopa might be a predisposition to cleptoparasitism, as the females are collecting pollen with reduced efficiency. This links back to the discussion brought forth in the review by Wcislo et al. (2004b) that proposes gynandromorphy as a potential evolutionary pathway to cleptoparasitism, more on this in the circadian rhythm section below. The relatively greater proportion of the sterna covered with female-typical hairs suggests that bilateral gynandromorphy need not be symmetrical. The asymmetric sterna and sting suggest that the internal anatomy of our specimen may also be of mixed sex, but we did not dissect it. Furthermore, external and internal anatomy do not always correspond in gynandromorphs (Ugajin et al. 2016).

Hinojosa-Díaz et al. (2012) showed that there is a Holarctic bias in gynandromorph collection, but our study and other recent works (Alvarez et al. 2014, 2019, Prashantha 2019) show that increased research attention in other parts of the world is leading to further discovery of gynandromorphs. Our study highlights a new occurrence of this rare phenomenon and calls for continuously close examination of the morphology of specimens to determine the frequency and distribution of gynandromorphs globally.

Circadian rhythm

This is the first time circadian rhythm was quantified in a gynandromorph and in any species of *Megalopta*. Both male and female *Megalopta* have a bimodal foraging period, with flight occurring approximately 90 minutes before sunrise and after sunset (Kelber et al. 2006). All three specimens used in this study were newly emerged with no prior exposure to natural light conditions, their activity over four days in darkness shows their endogenous circadian rhythm. It appears that the gynandromorph activity rhythm was shifted significantly earlier in the day compared to the male and female of *M. amoena*. In terms of the percentage of activity across the day, the gynandromorph was intermediate between the male and female. In terms of activity intensity, the gynandromorph was more similar to the female than the male. It is possible that its high intensity was due to the gynandromorph trying to express an activity typical of a female *Megalopta*, like foraging for pollen. However, the time shift was atypical and it

seems that though the gynandromorph may have presented an intact neurological circuit for foraging, it also had a delay in conducting that activity. We are referring to this anomalous activity of the gynandromorph as a deviant activity pattern. The name deviant activity pattern is based on the term pheno-deviant used by Wcislo et al. (2004b) to describe deviant phenotypes such as bilateral gynandromorphy. This pattern could result from having a split-sex brain (Matsuo et al. 2018). Future studies of organisms with deviant phenotypes may find this pattern consistent.

To derive meaning from the deviant activity pattern in the gynandromorph we revisit the hypothesis that gynandromorphs could be a potential evolutionary precursor to cleptoparasitism; see Wcislo et al. (2004b) for a review on this hypothesis. In an observational study of *Neopasites cressoni* Crawford 1916, a cleptoparasite of ground nesting bees, the cleptoparasite began flying as early as their hosts and continued after the host ceased (Torchio et al. 1967). Similarly, the gynandromorph *M. amoena* is active when the female has ceased. Our data is not sufficient enough to support that gynandromorphs are the ancestral state of cleptoparasites, rather the deviant activity pattern reported here suggests that there may be a mechanism related to gynandromorphy that might help explain that evolutionary path.

Previous behavioral observations of gynandromorph bees include some instances of individuals conducting behavior characteristic of one sex despite their mixed phenotype, such as excavating a nest or collecting pollen, both female traits (Michez et al. 2009). However, in other cases gynandromorphs expressed novel behaviors. For instance, a gynandromorph of *Bombus ignitus* (Smith, 1869) with a bilaterally split head and male genitalia approached virgin females in a lab assay but took much longer than male bees to attempt copulation, and never successfully copulated (Matsuo et al. 2018). A different gynandromorph of *B. ignitus* also showed non-functional courtship behavior and different levels of expression of the gene *doublesex* between male and female tissue (Ugajin et al. 2016). A gynandromorph of *Osmia ribifloris biedermannii* Michener 1936 with a male head and female body attracted male bees that attempted to mate with it, but none were able to copulate (Sampson et al. 2010).

Although we did not measure gene expression or dissect the brain, our circadian rhythm results suggest a situation potentially similar to the gynandromorph *B. ignitus* described by Matsuo et al. (2018): a bilaterally split brain unable to integrate conflicting sex-specific signaling. However, it should be noted that ours was only a single sample. More studies need to be done to better understand if there is a difference in circadian rhythm based on sex in this species, and to distinguish what the deviant activity pattern of the gynandromorph results from.

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